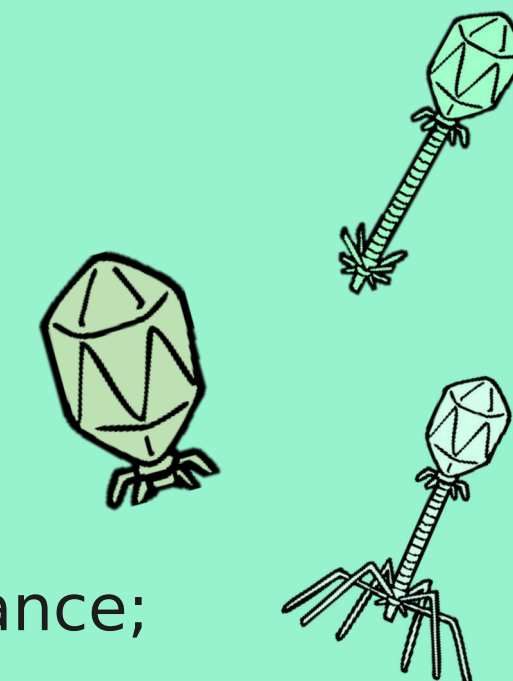


Spotlight on the viral diversity of fermented vegetables using viral metagenomics

Julia Gendre¹ (julia.gendre@inrae.fr), Claire Le Marrec², Stéphane Chaillou³, Sophie Landaud¹, Eric Dugat-Bony¹

¹ Université Paris-Saclay, INRAE, AgroParisTech, UMR SayFood, Palaiseau, France; ² UMR 1366 Cœnologie, INRAE, Univ. Bordeaux, Bordeaux INP, France; ³ Université Paris-Saclay, INRAE, AgroParisTech, Micalis Institute, Jouy-en-Josas, France

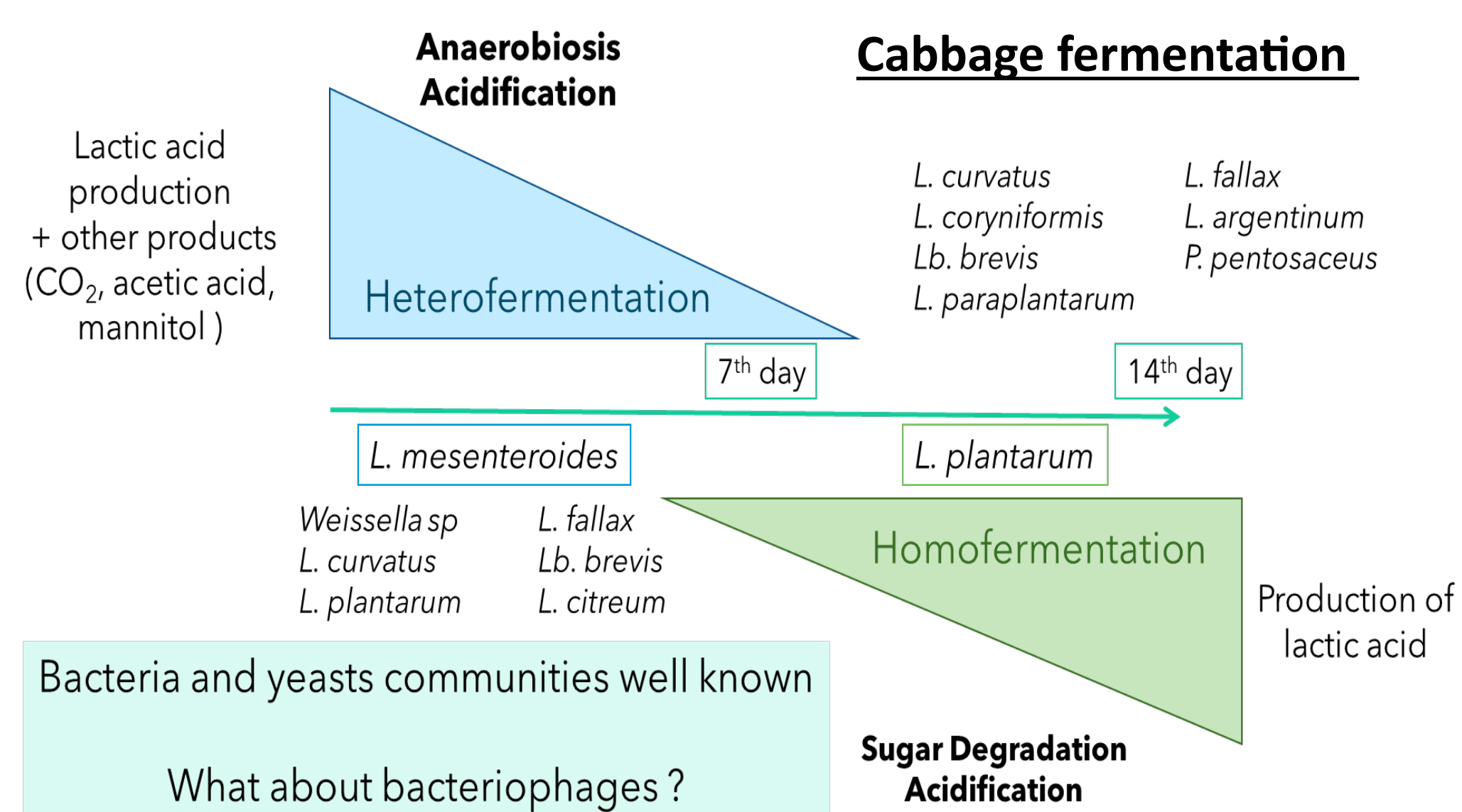


Introduction

Fermented vegetables are usually produced using the natural microorganisms present in the raw material. The process is characterized by a first heterofermentative step, generally carried out by *Leuconostoc mesenteroides*, which acidifies the medium and creates anaerobic conditions. The second homofermentative step is mainly carried out by *Lactiplantibacillus plantarum*. The composition of bacterial and fungal communities present in fermented vegetables (cabbage, carrot, turnip) have been widely characterized, whereas only few studies described the viral communities of these ecosystems.

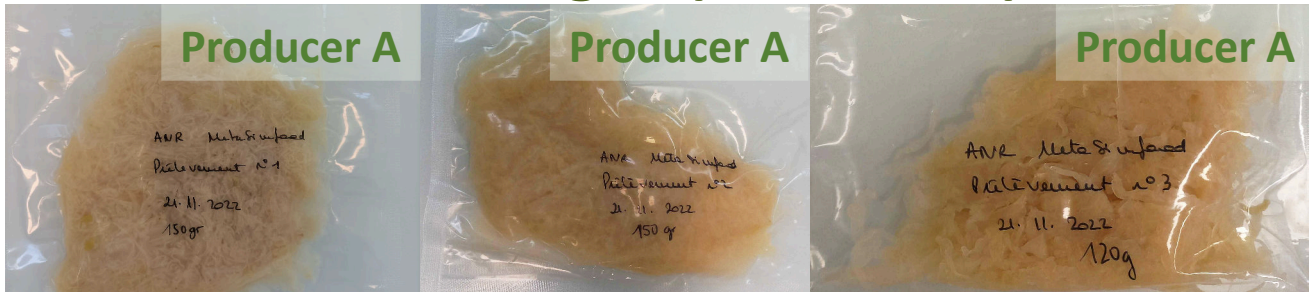
Several phages infecting different bacterial species were previously isolated from sauerkraut during the fermentation process, which revealed for the first time the complex ecology of phages in this ecosystem¹. Later, viral metagenomic analysis performed on different fermented foods including sauerkraut and kimchi highlighted again that complexity and showed that the vast majority of the sequences originated from uncharacterized phages².

The objective of the present study was to describe the viral composition of commercial fermented vegetables (sauerkraut, fermented carrots and fermented turnips) as a first stone to build future research on the phage ecology in this peculiar ecosystem.



Material

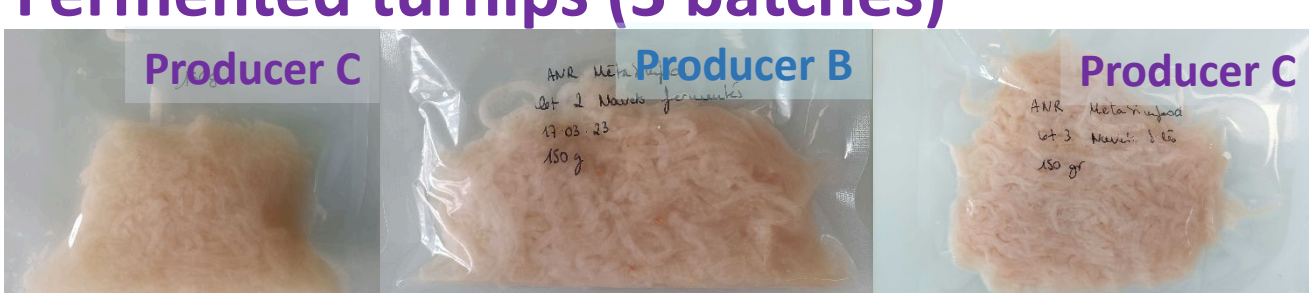
Fermented cabbages (3 batches)



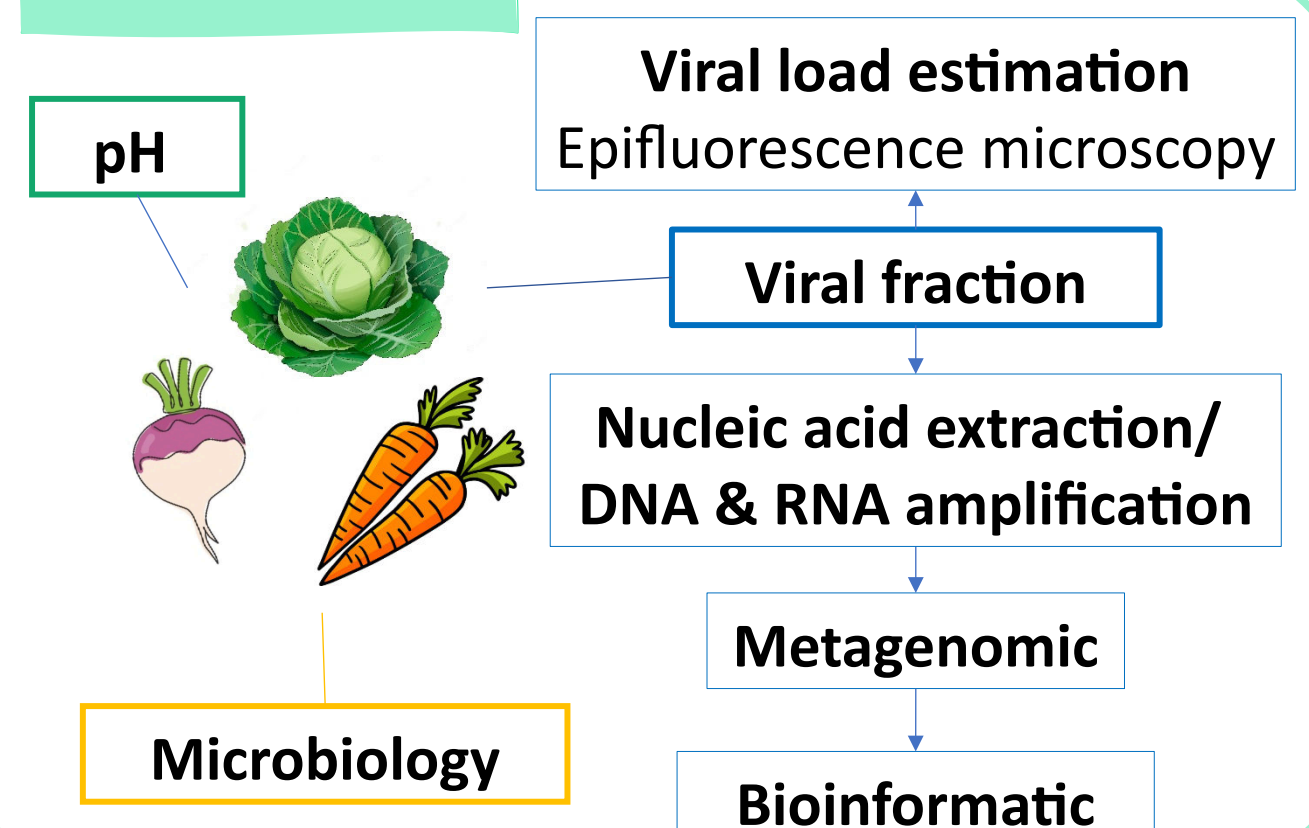
Fermented carrots (3 batches)



Fermented turnips (3 batches)

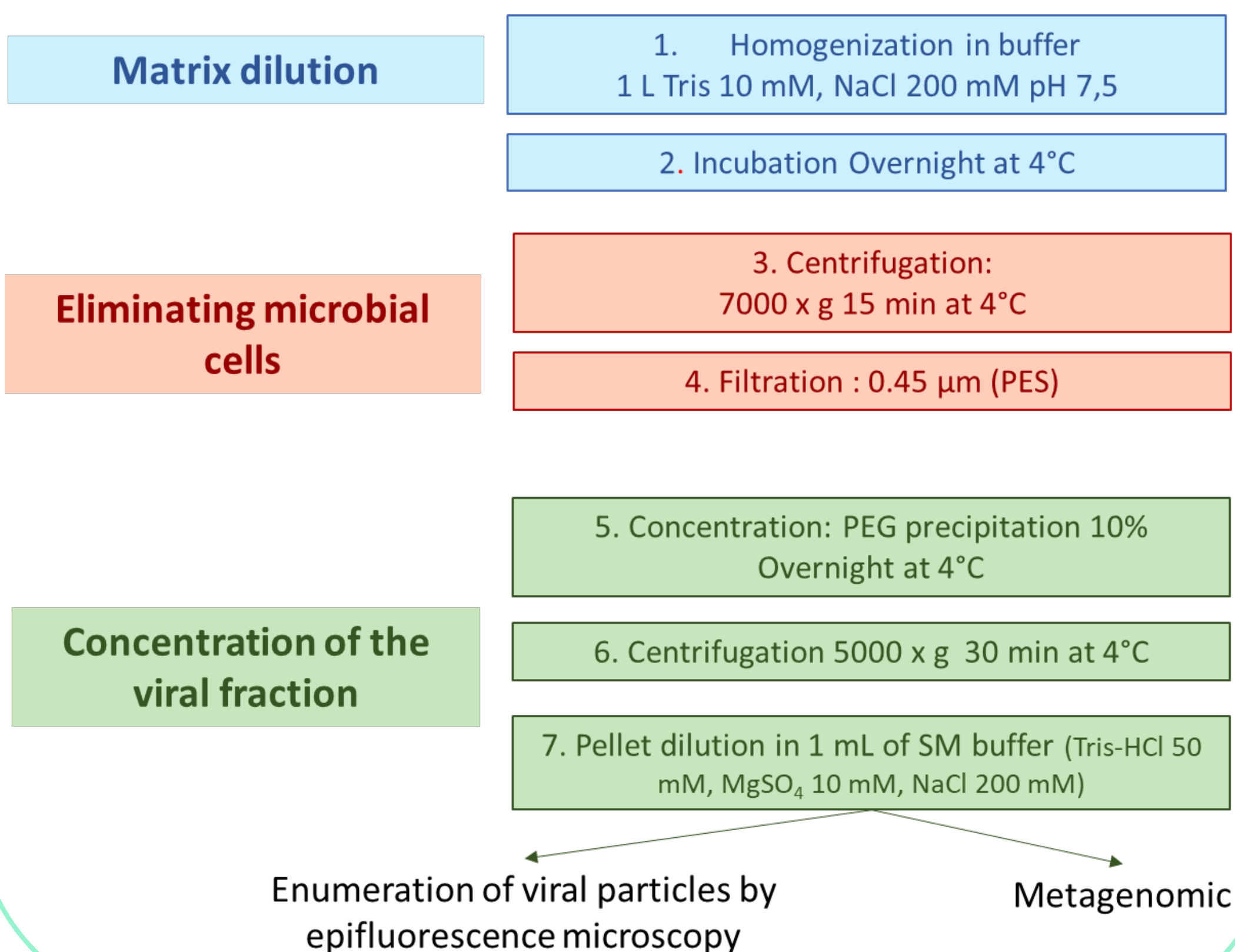


Method

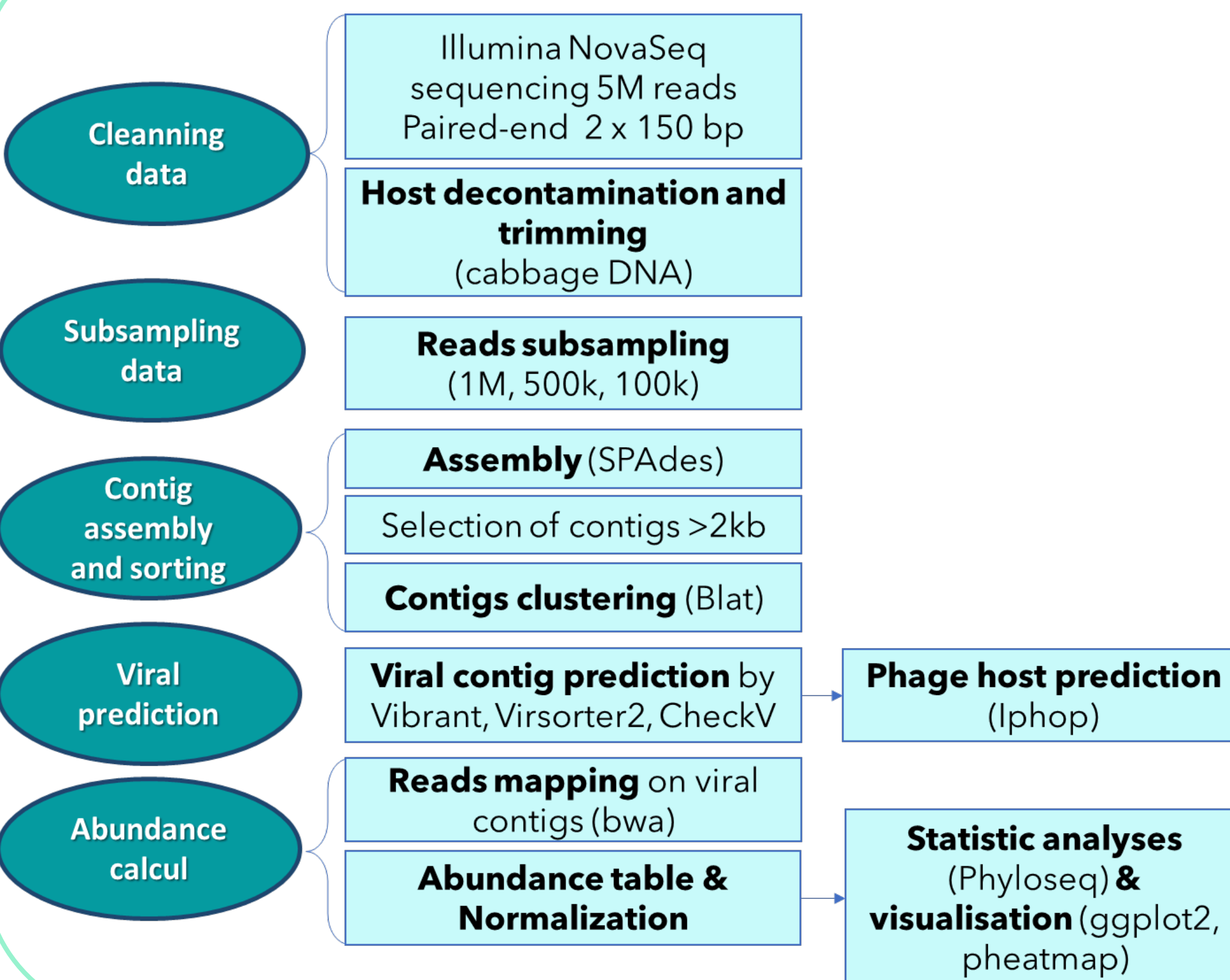


Viral particles extraction method

Extraction and concentration of viral fraction from 10 g of fermented vegetables



Bioinformatic pipeline



Epifluorescence microscopy

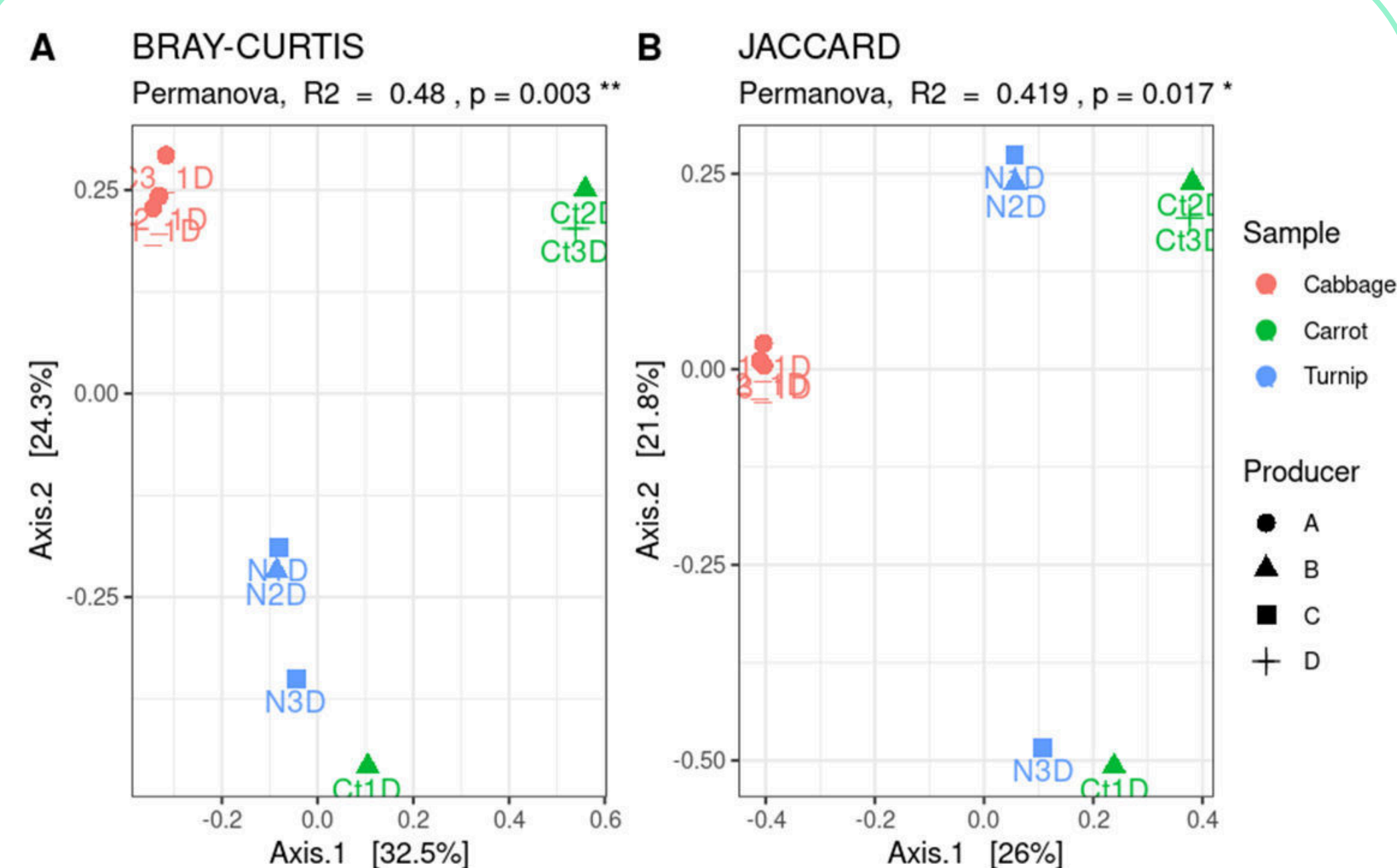
Epifluorescence microscopy - viral load estimation	Viral load estimation (phages/mL)
Cabbage batch 1 (pH 3,39)	2.14E+08
Cabbage batch 2 (pH 3,36)	1.02E+08
Cabbage batch (pH 3,53)	4.47E+07
Carrot batch 1 (pH 3,34)	6.16E+07
Carrot batch 2 (pH 3,84)	1.43E+08
Carrot batch 3 (pH 3,47)	2.70E+07
Turnip batch 1 (pH 3,67)	1.27E+08
Turnip batch 2 (pH 3,55)	6.93E+07
Turnip batch 3 (pH 3,30)	NA
Control buffer	4.01E+04

Viral metagenomic data

Samples	No. Contigs assembled >2kb	Longest contig	Final dataset		
			No. Contigs > 2kb	No. Clustered contigs	No. Predicted viral contigs
C1_1D	308	31 kb	2832	1293	541
C2_1D	35	27 kb			
C3_1D	295	46 kb			
Eau_CD	307	5 kb			
Tampon_CD	219	8 kb			
N1D	611	33 kb			
N2D	194	18 kb			
N3D	0	-			
Ct1D	21	3 kb			
Ct2D	169	49 kb			
Ct3D	40	10 kb			
WD (water control)	11	2 kb			
BD (buffer control)	179	6 kb			

N3D and Ct1D samples: low sequencing quality and poor assembly statistics compared to other samples.

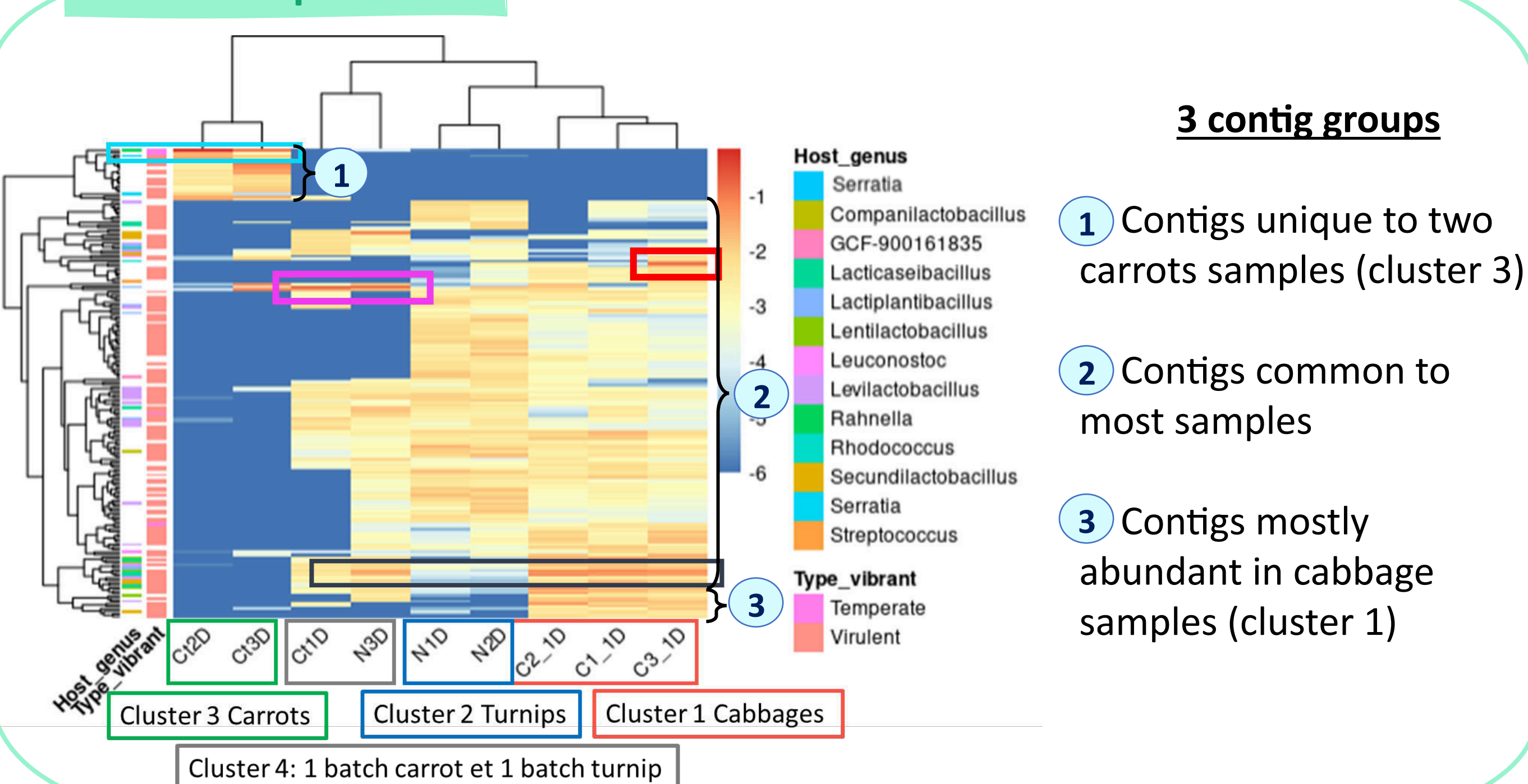
Virome beta-diversity



- The first axis helps discriminating samples according to the vegetable
- Samples N3D and Ct1D cluster together (low quality samples)

Viral composition depending on the plant matrix

Viral composition



3 contig groups

- 1 Contigs unique to two carrots samples (cluster 3)
- 2 Contigs common to most samples
- 3 Contigs mostly abundant in cabbage samples (cluster 1)

Most abundant viral contigs

Contigs identity	Infection cycle	Contig quality	Host order	Host family	Host genus	score_iphop
Ct1D_125k_NODE_1_length_2064	-	-	Lactobacillales	Lactobacillaceae	Lactiplantibacillus	94.0
Ct2R_125k_NODE_1_length_41237	Temperate	High quality	Enterobacteriales	Enterobacteriaceae	Rahnella	91.4
C1_1D_25k_NODE_2_length_23337	Virulent	low quality	Lactobacillales	Lactobacillaceae	Lentilactobacillus	91.4
Ct2D_250k_NODE_59_length_2554	-	-	-	-	-	-
C2_1D_250k_NODE_1_length_27078	Virulent	high quality	Lactobacillales	Lactobacillaceae	Lactocaseibacillus	99.4
C3_1D_25k_NODE_2_length_16738	Virulent	medium quality	Lactobacillales	Lactobacillaceae	Lactocaseibacillus	99.4
N2D_125k_NODE_42_length_2495	-	-	-	-	-	-
C3_1D_25k_NODE_3_length_5026	Virulent	low quality	Lactobacillales	Lactobacillaceae	Lentilactobacillus	91.1
C2_1D_250k_NODE_2_length_13954	-	-	-	-	-	-
C1_1D_25k_NODE_1_length_31726	Virulent	high quality	Lactobacillales	Lactobacillaceae	Lactocaseibacillus	96.6
C3_1D_125k_NODE_58_length_3209	-	-	Lactobacillales	Lactobacillaceae	Lentilactobacillus	91.6

The most abundant contigs are different between samples. The hosts for these contigs, when predicted, correspond to bacteria usually found during the late stage of the fermentation process. Phages infecting *Leuconostoc* were also detected but at a lower abundance (not present in the top 10)

Conclusion and perspectives

This study provides an overview of the composition of viral communities in different commercial fermented vegetables, with a particular focus on extracellular particles (virion forms). We identify phages in high abundance that infect bacteria belonging to genera found in the late stage of the fermentation process (e.g. *Lactiplantibacillus*, *Lentilactobacillus*,...), and at a lower abundance phages infecting bacteria of the early stage (e.g. *Leuconostoc*) or spoilage bacteria (e.g. *Enterobacter*). Our results suggest that several phages infect most of the dominant lactic acid bacteria involved in the fermentation process and, therefore, may impact the bacterial successions occurring in this system. Since phages are likely to modify fermentation kinetics and consequently the quality of end-products, it is now necessary to better understand their individual ecological roles and ultimately set-up technological strategies to better control their activity and secure fermentation.

1 Lu et al., *Bacteriophage Ecology in Commercial Sauerkraut Fermentations*, 2003, DOI: 10.1128/AEM.69.6.3192-3202.2003

2 Park et al., *Metagenomic Analysis of the Viral Communities in Fermented Foods*, 2011, DOI:10.1128/AEM.01859-10

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